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ON CHANGES IN NUMBER OF THE ANTERIOR HORN CELLS OF THE SPINAL CORD IN THE POLYDACTYLISM MICE

by

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INTRODUCTION

The number of the nerve cells in the anterior horn of the spinal cord in the animals with polydactylism, was examined by Tsang (1939) on the adult "Fortuyn" mice and by Baumar & Landauer on the adult fowls of "Houdan" strain. They equally noticed the increase in cell number in the retrodorsolateral column and dorsolateral column of the spinal cord on the same side of the polydactylism, as compared with those on the opposite side.

According to the Nishimura's method, we injected 10% ethylurethane solution into the intraabdominal cavity of the pregnant mouse on the 10th pregnant day and succeeded to produce polydactylism fetuses, either bilaterally or just on the right side. On these polydactylism fetal mice we examined the cell number of the main lateral nuclei in the anterior horn of the spinal cord and compared with those in the normal fetuses.

MATERIAL AND METHOD

a) Material

Pregnant mice of the hybrid strain were injected with the 10% ethylurethane solution intraperitoneally in dose of 1.2 mg per gram body weight on the 10th day of pregnancy. On the 19th pregnant day, laparotomy was carried out to take the fetuses out of the mother mouse. Through this procedure, 3 bilateral polydactylism fetuses and 5 with polydactylism on the right side were obtained.

These 8 polydactylism fetuses together with 5 normal control fetuses were examined in the present study.

The body length of the 19th day fetal mice is approximately 2.0～2.5 cm and it is impossible to take out the spinal cord from the vertebral canal in such a small animal. Thus, the fetuses were fixed in toto with 10% neutral formalin solution, and embedded in celloidin. Serial cross sections through the trunk in 15 μ thickness were made. Sections were stained with 0.02% thionin solution.

b) Method of Counting

Before counting the cell number of the anterior horn nuclei, there are two problems to be solved. First, how to decide the level of the lumbosacral segment of the spinal cord, which really innervates the lower extremity, particularly the 1st toe.

And second, what part of the spinal cord at that particular level should be chosen for actual counting to compare the cell number on one side to that on the other. From the reason which will be mentioned later, however, it was thought to be most reasonable, to pick up the one section at the lumbosacral level in which the total cell number in the main lateral cell groups, i. e. ventrolateral, dorsolateral and retrodorsolateral column of the anterior horn nuclei, was the largest as compared to others, and then to take out 30 consecutive sections, including this particular one in the center (Figs. 1 and 2). These 30 sections were further devided into 6 sets containing 5 sections in each. The numbers shown in the figures are the sum of cells in each set.

The total number of the nerve cells of those 6 sets on one side was compared with that on the opposite side.

NORMAL HISTOLOGY

Anterior horn cells of the spinal cord in the 19th day fetal mice were larger in size as compared to others. They were polyhedral and contained heavily stained NISSLE's granules and a pale nucleus. They were easily distinguishable from other kinds of nerve cells.

Although these anterior horn cells varied considerably either in their arrangement or number at the different spinal cord levels, they were roughly divided into 4 cell groups. According to the nomenclature of human anatomy, they may be called (1) ventromedial column (2) ventrolateral column (3) dorsolateral column and (4) retrodorsolateral column. Though there, of course, was some variety in the cell arrangement in each individual, following findings were common.

1) Ventromedial column

This column is situated at the ventromedial margin of the anterior horn. Cells in this column are rather small in size and long axis of the cell runs parallel to the margin of the gray matter. Number of the nerve cells is fewer than that of other columns. This column is to be seen in the lumbosacral segments of the spinal cord, but sometimes it is discontinuous. Since it has been generally considered that the nerve cells of this column innervate the abdomen and trunk, they were omitted from counting in the present study, because in this experiment, nerve cells which innervate the lst toe were to be discussed.

2) Ventrolateral column

This column occupies the ventral and outermost part of the anterior horn, and the number of cells is relatively small. It is found in the whole lumbosacral segments of the spinal cord. Nerve cells are large and polyhedral, composing cell column of different shape.

In the cross sections made through the level of the upper pole of the kidney, the upper lumbar segment of the spinal cord is to be seen. At this level the cell group of the ventro-lateral column is oval in shape. Coming downwards, it becomes gradually round, and at the level where the dorsolateral column begins to appear, the cell number of the ventrolateral column becomes constant. Sometimes these two

columns fuse with each other and the boundary between the two becomes obscure.

Since it is generally believed that the nerve cells in this column innervate the trunk and pelvis, it would be wise to omit this from the counting. However, from the reasons, that the boundary of this column to the dorsolateral column is not always clear, and that the cell number of the column is rather constant and not so many, this was included in the main lateral cell groups.

3) Dorsolateral column

This column is situated dorsally to the ventrolateral column. Nerve cells are large and polyhedral, and form the cell group of the elongated shape in the ventrodorsal direction. Number of the nerve cells in this column is the largest among the columns of the main lateral cell groups. This column begins to appear in the sections through the level between the upper and the middle third of the kidney. In the sections at the level of the renal pedicle the number of cells decreases markedly and the cellular density becomes small.

At the level between the upper and the middle third of the kidney this column sometimes fuses with ventrolateral column, and at the level between the middle and the lower third of the kidney, it fuses with retrodorsolateral column. Sometimes this column is composed of four or five small subgroups.

The number of the nerve cells of this column was counted, because these cells have been considered to innervate the lower extremity.

4) Retrodorsolateral column

Nerve cells in this column are little bit smaller than those of dorsolateral column and the cell number is not so many. This column is situated most dorsally and found in the sections from the level of the renal pedicle to that between the middle and the lower third of the kidney. It sometimes fuses with the dorsolateral column.

Box considered that the lst toe was innervated by the nerve cells which were situated most dorsally in this column. Thus, this column is believed most important for the purpose of the present study.

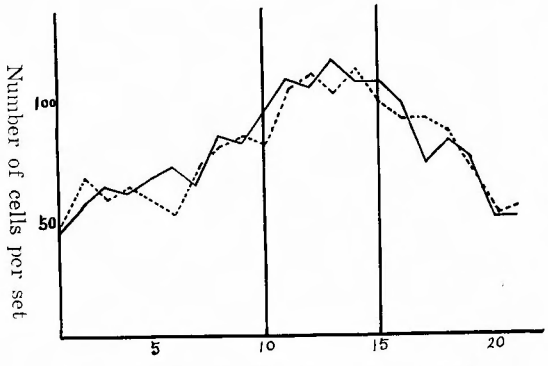
At any event, it seems reasonable to assume that the columns which innervate the distal parts of the lower extremity are the dorsolateral and retrodorsolateral column. The total number of the nerve cells in these two columns is rather very large as compared with that of the anterior horn cells in the ventrolateral column.

RESULTS

1) Normal fetal mice [Figs. 1 (N2), 2 (N3-1), 3 (N3-2), 4 (N4) and 5 (N5)]

5 normal fetal mice were used for the control. The cell number of the main lateral cell groups was calculated on the right and left side separately. Total number of the cells on one side in one set of sections i. e. in 5 consecutive serial sections, was graphically illustrated in Figs. 1, 2, 3, 4, 5. In 4 cases out of 5 (Figs. 1, 2, 4, 5), curves were of essentially the same pattern and showed 2 peaks (one large and one small). The first small peak corresponded to the level at which the upper pole of the kidney did appear, and ventrolateral and dorsolateral columns were seen.

Fig. 1 (N 2) — R ··· L



No. of sets (5 slides in each one set)

Fig. 2 (N3—1) — R ··· L

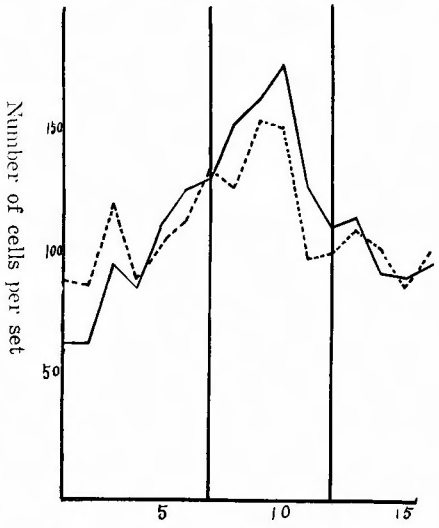


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Fig. 3 (N3—2) — R ··· L

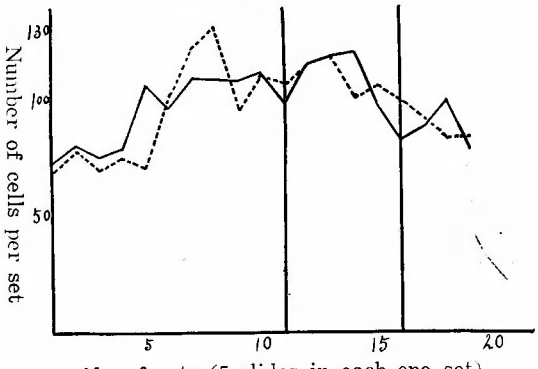

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Fig. 4 (N 4) — R ··· L

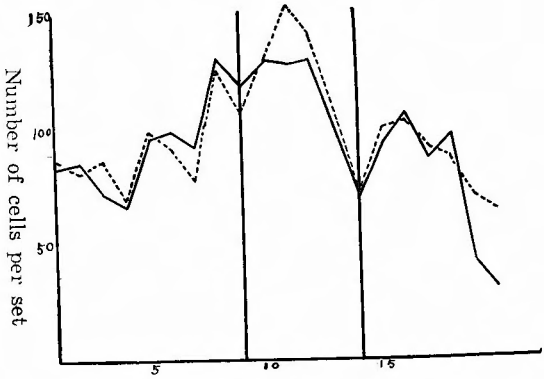

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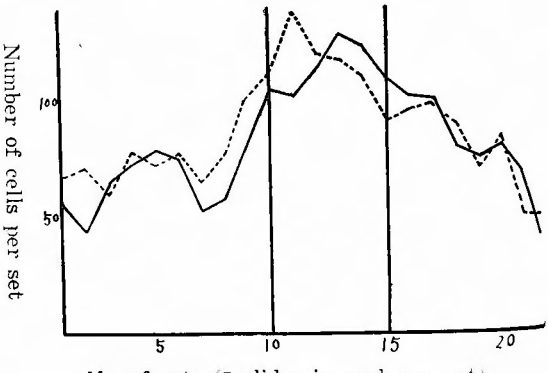
Fig. 5 (N 5) — R ··· L



No. of sets (5 slides in each one set)

Then the curve was directed downwards. This was due to the local decrease of the cell number in the dorsolateral column. Again, however, the cell number increased and the curve was directed upwards. It continued to rise beyond the height of the first peak. This was the beginning of the second large peak and at this level a part of retrodorsolateral column began to be seen. Thus, it was clear that the second peak was made up of the cells mainly of the dorsolateral and retrodorsolateral column.

At the level which corresponded to the top of the second peak, it was found that the retrodorsolateral column developed in the highest degree without any exception.

Fig. 6 (M 11) — R ··· L

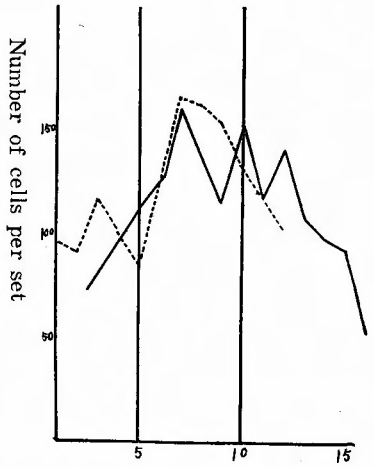


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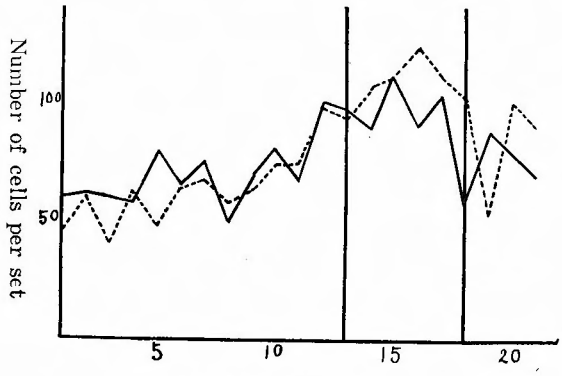
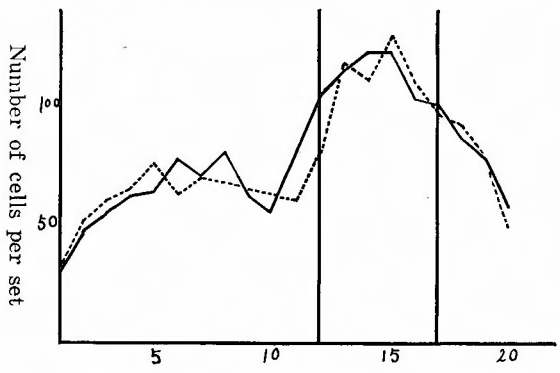
Fig. 7 (M 12) — R ··· L



No. of sets (5 slides in each one set)

Fig. 8 (M 13) — R ··· L



No. of sets (5 slides in each one set)

Table 1 Normal control

	R	L	
N 2	641 (51 %)	613 (49 %)	+28 (2 %)
N 3 (1)	856 (52.9%)	761 (47.1%)	+95 (5.8%)
N 3 (2)	630 (49.5%)	644 (50.5%)	−14 (1 %)
N 4	669(49 %)	695 (51 %)	−26 (2 %)
N 5	681 (49.5%)	695 (50.5%)	−14 (1 %)

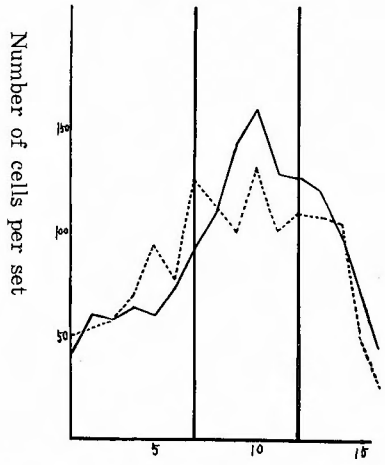
Table 2 Polydactylism of the bilateral lower limb

	R	L	
M 11	807 (49.7%)	819 (50.3%)	−12 (0.6%)
M 12	589 (47.6%)	649 (52.4%)	−60 (4.8%)
M 13	669 (50.9%)	646 (49.1%)	+23 (1.8%)

Table 3 Polydactylism of the right lower limb

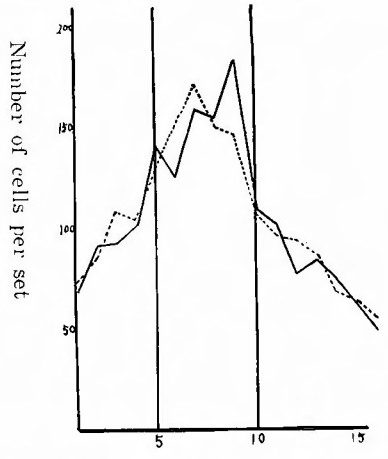
	R	L	
M 1	774 (53 %)	684 (47 %)	+90 (6 %)
M 2	869 (50.4%)	853 (49.6%)	+16 (0.8%)
M 3	824 (51 %)	788 (49 %)	+36 (2 %)
M 4	774 (51 %)	740 (49 %)	+34 (2 %)
M 5	620 (52 %)	562 (48 %)	+58 (4 %)

Fig. 9 (M 1) — R ··· L



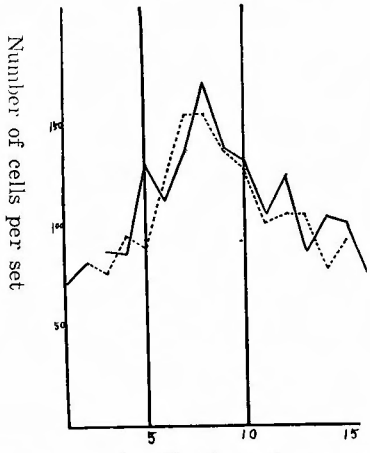
No. of sets (5 slides in each one set)

Fig. 10 (M 2) — R ··· L



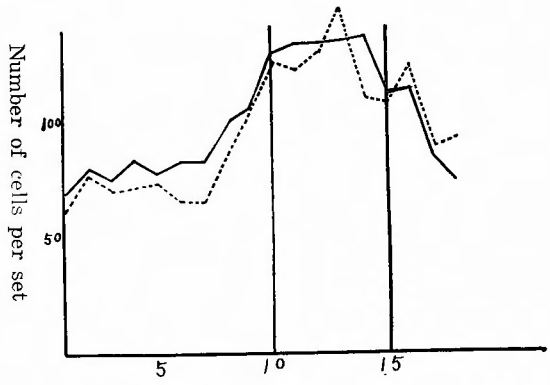
No. of sets (5 slides in each one set)

Fig. 11 (M 3) — R ··· L



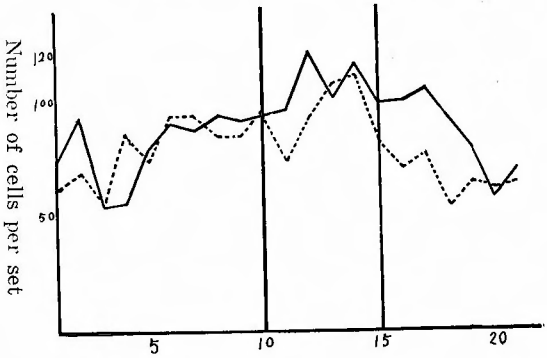
No. of sets (5 slides in each one set)

Fig. 12 (M 4) — R ·· L



No. of sets (5 slides in each one set)

Fig. 13 (M 5) — R ··· L



No. of sets (5 slides in each one set)

In the remaining one normal case the curve was slightly different from others (Fig. 3). It also demonstrated two peaks, but they were of aproximately the same height. At the level of the second peak retrodorsolateral column did also appear but the increase in cell number was not so marked.

Then, as mentioned before, total number of cells in 30 consecutive sections including the one, which corresponded to the top of the second peak, in center, was calculated on each right and left side, and compared with each other. In consequence of this calculation it was revealed that even in the normal animals there was some difference between the cell numbers on both sides. That is, in 4 cases out of 5 the side-difference fell in a range between 0.6 to 2%, but in one it reached as large as 5.8% (N3-1) (Table 1).

2) Fetal mice with bilateral polydactylism [Figs. 6 (M-11), 7 (M-12), 8 (M-13)]

For these malformed fetal mice the same counting method as for normal control

fetal mice was adopted. Also the total number of cells in the 30 consecutive sections, centering the second peak among them, was calculated on either side to compare one with the other.

It was revealed that in the fetuses with bilateral polydactylism, the second peak was not necessarily higher than that in the control fetal mice. And its percentage was in M-11, 49.7% on the right side, 50.3% on the left, in M-12, right 47.6%, left 52.4% and in M-13, right 50.9%, left 49.1%, and the side-difference was 0.6% 4.8% and 18% respectively (Table 2).

3) Fetal mice with right-side polydactylism [Figs. 9 (M-1), 10 (M-2), 11 (M-3), 12 (M-4), 13 (M-5)]

In the 5 fetal mice with right-side polydactylism the cell number around the second peak was larger on the right side than that on the left. Its percentage was in M-1, right 53%, left 47%, in M-2, right 50.4%, left 49.6%, in M-3, right 51% left 49%, in M-4, right 51% left 49% and in M-5, right 50% and left 48% (Table 3). The side-difference in these mice was 6, 0.8, 2, 2 and 4% respectively. These results might give an impression that the existence of the polydactylism might cause a change in number of the anterior horn cells. Through the statistical study, however, those differences were not significant under the risk level of 5%.

DISCUSSION

Tsang, counting the anterior horn cells in the adult mice with polydactylism of the Fortuyn strain, found the increase in cell number or hyperplasia of the retrodorsolateral column on the same side of the polydactylism. He considered that some unknown factors acted upon this cell column and gave rise to the polydactylism.

Baumann and Landauer, on the other hand, after the similar study on the spinal cord of the fowls of the Houdan strain, considered that the inborn malformation or asymmetry of the peripheral organ influenced the spinal motor nurons and caused the difference of the cell number on both sides.

At all events as far as the results of the present experiment were concerned, there was no such close correlation between the existence of polydactylism and the number of the anterior horn cells as reported by Tsang and Baumann.

However, Allen (1912) stated that in rats cell division of the spinal cord continued through its whole intrauterine life. Also according to Gonzales, in the anterior horn cell groups of the cervical cord of the newborn rats, the fusion of different columns or the subdivision of each column did take place, which had never been observed in the adult animals.

Thus, there might be some difference in the results between our experiment in which the 19th day fetal mice were used and Tsang's experiment in which the adult mice were used. However in our present experiment, the cell division of the anterior horn had never been observed.

In our materials, we also could observe the fusion or subdivision of the cell columns as Gonzales did, and found difficulty in counting the cell number of each column separately. Consequently, we had to count the cell number of the whole

main lateral cell groups on either side to compare each other. In the main lateral cell groups, the ventrolateral column which had been believed to have nothing to do with the innervation of the lst toe of the lower limb, was also included. Thus, the counting of these cells together, may cause some difference in results from that by TSANG et al.

However, if the counting of the cells in the ventrolateral column comes into question, the counting of the cell number in the dorsolateral and retrodorsolateral columns may also be criticized, because these columns extend for a considerably long distance from the lower lumbar to the upper sacral segments, and therefore it is not likely that the polydactylism in which only the 1st toe is divided, may give rise to such a marked side-difference in cell number of these widespread columns. In case that there might be a marked difference in the total cell number in these entire columns between the right and the left side, there would be the gross asymmetry of the whole lower limbs. In fact, in the fetal mice with polydactylism in our experimental series, such a high degree of anomaly in the lower limb was not observed.

And, as far as the 19th day fetal mice, which had been treated with urethane, were concerned, no marked change in number of the anterior horn cells in the lumbosacral segments on both sides was found.

CONCLUSION

The 19th day fetal mice with polydactylism in the lst toe of the lower limb, which had been received urethane injection during their intrauterine life, were used for the present study.

Among the anterior horn cell columns of the lumbosacral segments, the cell number of the main lateral cell groups was counted on either side and compared with each other.

The results was that there was no statistically significant difference in cell number between two sides under a risk level of 5%.

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和 文 抄 録

多趾症マウスの脊髄前角細胞の数的変化に就いて

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山 崎 徳 雄

1939年 Tsang は Fortuyn 株の多趾症マウスに就いて，又1943年 Baumann 及び Landauer は Houdan 株の成熟鶏の多趾症に就いて脊髄前角細胞数を算定し，多趾の存在が，同側脊髄前角細胞数と密接な関係があるのを認めた．私は西村氏法によつて妊娠第10日目のマウス母体腹腔内に10%エチールウレタンを注入して後，胎生19日目に取出した胎仔に両側又は右側の多趾症を得たが，之等多趾症を有する胎仔の前角核中外側主群について細胞数を算定し正常胎仔と比較して如何なる左右差があるかを見た．

その結果は次の通りである．

1) 正常例（5例）

正常例であつても可成り個体差が認められる．左右差を百分比で示すと N2：右51%左49%，N3-1：右52.9%左47.1%，N3-2：右49.5%左50.5%，N4：右49%左51%，N5：右49.5%左50.5%であつて，その差は大多数が1%から2%の間であるが，最も差の大きいものは5.8%のものもある．

2) 両側性多趾症例（3例）

両側に多趾が存在しても対照例と比較して脊髄前角細胞数の増加が特に著明であることはなく，又左右の百分比では M11：右49.7%左50.3%，M12：右47.6%左52.4%，M13：右50.9%左49.1%であつてその差は各々0.6%，4.8%，1.8%であつた．

3) 右側多趾症例（5例）

右側のみに多趾を有する5例では，全例に於て多趾と同側の右側前角細胞数が左側より多い．左右の百分比は M1：右53%左47%，M2：右50.4%左49.6%，M3：右51%左49%，M4：右51%左49%，M5：右52%左48%で，その差は各々6%，0.8%，2%，2%，4%であつた．この結果から見ると多趾の存在が前角細胞数に多少の変化を与えるかの如くにも見えるが，推計学的検討を加えた結果5%以下の危険率に於て有意の差のないことが判明した．